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Improving meridic diets for laboratory rearing of the European corn borer

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IMPROVING MERIDIC DIETS FOR LABORATORY REARING OF THE EUROPEAN CORN BORER

by

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INTRODUCTION

The expanded use of insects as laboratory animals and the growing emphasis on insect control by means of biological methods have increased the need for a non-seasonal supply of insects. Some of the biological control methods such as the use of ionizing radiation, sex sterilants, and the dissemination of disease organisms are under investigation as possible controls of the European corn borer. Existing methods of rearing the larvae of this insect on green plant material and on artificial media have been inadequate for producing large numbers of larvae economically. Since most of the programs on host plant resistance, biological and chemical control require large numbers of vigorous disease-free populations, the development of satisfactory growth media and procedures was undertaken. The three main objectives of this investigation were: (1) To modify existing diets and to employ one in a continuous rearing program, (2) to determine growth responses of the larvae after being reared continually on a meridic media for several generations, and (3) to develop a more precise chemically defined diet.

REVIEW OF LITERATURE

Historical

The European corn borer (Ostrinia nubilalis [Hbn.]) was probably introduced into the United States sometime between 1909 and 1914 in broomcorn imported from Hungary or Italy (Smith 1920). In a review of foreign literature, Caffrey and Worthley (1927a) reported it to be a pest in Europe of several crops other than corn. Vinal (1917) reported the first identification in the United States in 1917. The geographical distribution of the borer spread rapidly in the United States. Workers in New York and Pennsylvania reported infestations in 1919, and severe losses occurred in Ontario in 1925 and 1926. It was later reported by Caffrey and Worthley (1927b) that the borer was fairly well established in eastern Michigan and eastern Indiana. The spread was rapid west and south after 1936. In 1942 the borer had reached eastern Iowa. It was established over the western part of the Corn Belt by 1948 and reached the Rocky Mountains by 1950. According to Iverson (1957), 37 states of the Nation were infested with the corn borer in 1956 ranging from northern Mississippi and Alabama in the south to Canada in the north, and from eastern Wyoming in the west to the Atlantic Ocean on the east. The most severe loss attributed to the corn borer occurred in 1949 when, according to Beck (1950), 318,819,000 bushels of corn valued at $349,635,000 were destroyed.

Biology

Several authors have reported on the biology of the European corn borer. Vinal and Caffrey (1919) described the life cycle and gave an

account of the feeding habits of the larvae on the corn plant in the New England area. The borers in this area were predominantly two brooded. Huber et al. (1928) published a detailed account of the life history in the one-generation area in Ohio. Goleman (1954) and Weekman (1957) have presented valuable information toward a better understanding of the ecology of the borer in Iowa.

According to Goleman (1954) and Weekman (1957) spring pupation in central Iowa occurs principally during the latter part of May and the first part of June. Emergence of first brood moths occurs about 10 to 14 days later and oviposition reaches a peak in the latter part of June. The eggs of the borer are laid on the underside of the leaf and hatching occurs about a week later, depending upon temperature. The larvae tend to survive or die on the plant on which they hatch. This observation was made by Beck (1956a) along with the conclusion that the orientation and feeding behavior of the European corn borer larvae on the corn plant are the result of the fulfillment of a negative phototaxis, a positive thigmotaxis, and a saccharotropism. Huber et al. (1928) also reported the borer to be positively saccharotropic. Barber and Dicke (1944c) concluded that the first requirement of the first brood larvae is the location of a moist feeding area. Corn plants attacked by this generation are usually young and rapidly growing, so the larvae find variable food characteristics, some of which meet their needs for optimum growth better than others. They found, as did Vinal and Caffrey (1919), Cox (1955), and others, that most of the feeding by first instar larvae on corn in the whorl stage of growth is in the moist area on the surface of the upper three or four unfurling leaves. Barber and Dicke (1944c) reported work that agreed with

preliminary studies made by Huber (1938), who reported that early instar larvae feed principally upon etiolated tissue, that availability of such tissue could very well be responsible for larval survival, and that primary feeding is on etiolated tissue above the moist area in the whorl. They also reported that under field conditions there is little feeding below the moist area located in the whorl. The habits of the second instar larvae are much the same as the first. With the emergence of the tassel, small borers move primarily to the tassel buds. According to Beck, in a Wisconsin Experiment Station bulletin compiled by Powers and Muchenhirn (1952), larvae, when given a choice, favored tassel bud tissue over leaf tissue. Hawkins and Devitt (1953) also stated that the young larvae feed upon the leaves in the whorl in an attempt to reach the developing tissue. Cox (1955) stated that when the borer reached the third instar they could be found tunneling into the midrib of the leaves, stalks, and ear shoots. Barber and Dicke (1944b) reported that nearly one-half of both fourth and fifth instar larvae were found burrowing into some part of the plant. However, most of the fourth instar larvae had not burrowed into the stalk, and over half of the fifth instar larvae had burrowed and tunneled into the stalk. Usually larval development is complete after five instars. Occasionally, however, as many as eight were observed. Vinal and Caffrey (1919) stated that the number of larval instars was dependent upon food supply.

The initiation of a second brood or generation of corn borers is dependent upon the physiological and inherent nature of the first brood of larvae. Bottger (1942) concluded that expression of a single or

multiple generation is probably influenced by nutrition. Arbuthnot (1944) stated that the development of a second brood is an inherent characteristic and is due to the fact that two genetic strains of borers exist. Vinal and Caffrey (1919) were among the first to report a second generation of borers. Fitch (1936) reported a second generation of borers in Indiana, and Vance (1939) reported an increase in the proportion of European corn borer producing two generations in the Lake States area.

When larval feeding terminates a light cocoon is spun. The larvae that pupate in mid-summer spin their cocoons in tunnels or externally upon the leaves. Goleman (1954) and Weekman (1957) have shown that summer pupation in Iowa occurs during the last of July and the first part of August. The moths emerge about 2 weeks later and oviposition is heaviest in the latter part of August. Eggs are laid on the underside of leaves, on the flag leaves of the ears, and occasionally on the stalks. The hatching period for second brood eggs is usually from 4 to 7 days, depending upon temperatures. Vinal and Caffrey (1919) reported that first and second instar larvae feed primarily in leaf axils and behind leaf sheaths. Barber and Dicke (1944a) and Cox (1955) stated that pollen is an important food for the establishment of the young larvae in these locations. The behavior of the latter instar larvae of the second brood is well described by Cox (1955). It is much the same as that of the first brood larvae as described by Barber and Dicke (1944c). That is, the fourth instar larvae migrate to various points on the plant and the fifth instar larvae are found boring primarily in the stalk, ear, shank, or cob. The larvae usually overwinter in tunnels in the stalk.

Caffrey and Worthley (1927b) list approximately 170 possible host plants of the corn borer. Some of them are more susceptible than others to attack. Dicke (1932) in a study of the host plants of the European corn borer in Michigan, found that the borer would sustain itself on a number of host plants, but that most infestations in crops other than corn were brought about by larval migration from corn.

Nutrition

Wigglesworth (1950), Trager (1953), Lipke and Fraenkel (1956), and House (1961) have adequately covered the literature pertaining to insect nutrition in general. Nutrition of the European corn borer has been studied rather intensively in order to add to the knowledge of host-pest relationships and to produce a laboratory method of rearing which could be used for continual and non-seasonal production of egg masses and larvae.

The first work pertaining to corn borer nutrition was concerned largely with field observations and studies. Polivka (1931) stated that physiological changes within the corn plant were a factor causing reduction in borer populations. Houser and Huber (1929) indicated that each larval instar had particular food requirements and that nutritional factors greatly affect the rate of larval survival and establishment. Meyers in 1930, as quoted by Cox (1955, p. 12) suggested that larval survival seemed to be correlated directly with morphological and chemical differences associated with differences in the relative stage of development of plants during the egg hatching and larval establishment. Barber and Dicke (1945) theorized that the tissue larvae feed upon indicate that nutritional needs change with the stage of growth of the larvae. Beard

and Turner (1942) implied that the more rapid development of borers in the tassel than those elsewhere suggested a nutritional effect. Barber and Dicke (1944c) also stated that when feeding upon pollen, as when feeding upon very young etiolated tissue, the rate of development of first and second instar larvae was very rapid. Bottger (1951) in an experiment designed to determine the relation of sugar and protein content of different parts of the growing plant to the nutrition of the corn borer, found that survival was low on internode tissue which had a high sugar content and a low protein content, and that it was high on leaf tissue which had a low sugar content and a high protein content. Beck (1956b) observed that larval requirements for dietary sugar and protein were correlated with the normal biology and feeding behavior of the borer on the corn plant. However, Turner and Beard (1950) theorized that high survival of larvae on early planted C103 might be explained by the high sugar content at this relatively early stage of growth. But Turner (1951) in an attempt to establish a relation between sugar content of the corn plant and infestation and survival of the European corn borer stated that there was no relation between sugar content and infestation, or sugar content and survival. Beck (1956b) stated that the requirement for glucose was negligible during early stages of growth, but high during later stages of growth of the larvae and the opposite was true for protein.

One of the first projects solely for the purpose of obtaining information about the nutritive requirements of the corn borer was devised and carried out by Bottger (1940). He concluded from a preliminary study that larvae developed better on materials rich in glucose than on materials high in either starch or sucrose. He also found that there were no

enzymes present in the digestive tract of the corn borers capable of splitting starch into sugars, however, both sugar and protein splitting enzymes were found. From this same study, some indication was obtained as to the importance of the moisture content and physical characteristics of the food on the feeding behavior of the borer. In a later paper Bottger (1942) indicated that he had formulated an agar-cellulose base synthetic diet on which as many as 36 percent of the larvae fed on the diet survived to maturity, all resulting pupae had a normal pupal stage, and all moths emerged normally. In all he tested 20 different formulated food media. From these tests he concluded that casein was superior to zein as a source of protein and that peptone employed as a supplement to either casein or zein appeared to stimulate both feeding and growth of the larvae. He also stated that even though fat requirements were not established, all satisfactory media contained some fat. He also included mineral salts in all media. Some indication was shown that vitamins A, B, and E are of nutritional value to the borer.

Beck et al. (1949) reported on a purified diet that they had developed which allowed apparent optimum growth of corn borer larvae. They concluded from their studies which lead to the development of the diet that: the supplementary effects of peptone (first reported by Bottger (1942)) could be duplicated by the addition of choline at the rate of 0.4 percent of the dry diet, casein was an adequate source of protein, the lipid requirements could be satisfied by corn oil and cholesterol, a 2 percent level of Wesson's salts seemed to fulfill the mineral ion requirement, brewer's yeast powder was a satisfactory source of vitamins, but a mixture of 10

B-vitamins failed to replace brewer's yeast powder in the diet, and finally that an unidentified green leaf factor present in corn leaves was required for optimum growth. Beck and Stauffer (1950) offered a slight modification to the diet published by Beck et al. (1949), in that corn oil was replaced by linoleic acid and A tocopherol. Beck (1953) in a paper devoted to the corn leaf factor indicated that the brewer's yeast in the diet could be eliminated by a mixture of vitamins consisting of choline chloride, thiamine, riboflavin, nicotinic acid, calcium pantothenate, pyridoxine, inositol, p-aminobenzoic acid, folic acid and biotin. Also, he concluded in this paper that the corn leaf factor was heat stable, acid stable, water soluble and dialyzable, and that it was not identical with thiamine, riboflavin, pyridoxine, pantothenic acid, nicotinic acid, choline, inositol, para-aminobenzoic acid, biotin, folic acid, vitamin B_{12}, sodium nucleate, adenine, ascorbic acid, or carnitine.

From the results of an experiment designed to help understand the feeding behavior of the borer, Beck (1956c) concluded that corn borer larvae tend to prolong their feeding in proportion to the dietary sugar concentration. Beck and Hanec (1958) reported that the average feeding duration of first instar larvae was increased by L-alanine, D-L-a-amino-n-butyric acid, L-serine, and L-threonine. Also that a negative effect on feeding was observed with L-tryptophan, L-arginine, and B-alanine. Little or no response was evident with D-alanine, D-serine, and D-threonine.

REARING METHODS

Handling of the Larvae

Larvae used in this study were the progeny of field-collected moths or larvae. The field-collected larvae were obtained in the fall, placed in a cold room at 45° F. and held there until after January 1 of the following year. Periodically, as adults were needed for oviposition, the larvae were removed from the cold room and placed in an incubator at 85° F. Here, the larvae pupated and the moths emerged, mated, and laid eggs. Egg masses were obtained in approximately 20 days after the larvae were removed from the cold room. The moths were handled by the method recommended by Dicke*. Upon reaching the blackhead stage the egg masses were placed in individual containers and allowed to hatch. The larvae were then placed on the diet within 1 hour after hatching. Fifty larvae were started on each diet. The larvae were reared in a constant temperature cabinet held at a temperature of about 85° F. and a relative humidity ranging from 65 to 75 percent. Most of the experiments were arranged in randomized complete block designs with four to seven replications depending upon the number of larvae available from each egg mass. This was done to remove the chance of variation due to genetic differences between egg masses. Twenty-five larvae from each treatment were weighed on the tenth day after being placed on the media. After weighing the larvae were returned to the vials to continue their development. Date of pupation and

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*Dicke, F. F., Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture. Handling moths for optimum oviposition. Private communication. 1960.

the percent pupation were recorded for all of the surviving larvae that were started on each diet. After pupating, the pupae were placed in oviposition cages where the moths emerged. Oviposition data were recorded. After the death of all adults in the oviposition cages, the moths were counted and sexed. Pupal mortality and the number of moths emerging improperly (failing to become free of the pupal case or wings not completely expanded) were also recorded. Thus the four criteria used to determine treatment effect were larval weight, length of larval period, number of adults emerging properly, and number of egg masses per female. The criterion considered to be most significant in most of the evaluations was larval weight.

Diet Preparation

The basic diet used in all of the studies is a modification of the one described by Beck et al. (1949), the composition of which is given in Table 1.

The method used to prepare the diet is also a modification of the one discussed by Beck et al. (1949). Briefly it is as follows. The agar and one-half of the water were placed in a beaker in a boiling water bath and stirred for 5 minutes. Then the remainder of the dietary ingredients were added and the resulting mixture stirred for an additional 15 minutes. After cooking, the hot liquid mixture was poured into an aspirator bottle and dispensed from there into the rearing vials. This was accomplished by attaching a 12-inch piece of rubber tubing to the bottom outlet of the aspirator bottle, elevating the bottle, and then allowing the diet to move into the vials by gravity flow. However, for most diets, air pressure was

Table 1. Composition of European corn borer diet

	Amount used	Dry diet
	gm.	Percent
Carrier:		
Distilled water	255.00[a]	
Bacto-Agar	6.60	12.4
Glucose	10.50	19.8
Casein, Vitamin Free[b]	10.50	19.8
Cholesterol	0.85	1.6
Corn oil containing 1% Alpha tocophorol	0.50	1.0
Salts Mixture No. 2, U.S.P. XIII[b]	1.30	2.4
Choline Chloride	0.12	0.2
Brewer's Yeast U.S.P.	6.90	13.0
Leaf Factor	13.80	26.0
Mold Inhibitor Mixture:[c]		
n-Butyl p-hydroxybenzoate	0.6	1.1
Sorbic acid	1.5	2.8

[a]Plus 10 percent to compensate for evaporation during cooling

[b]Nutritional Biochemicals, Inc., Cleveland, Ohio

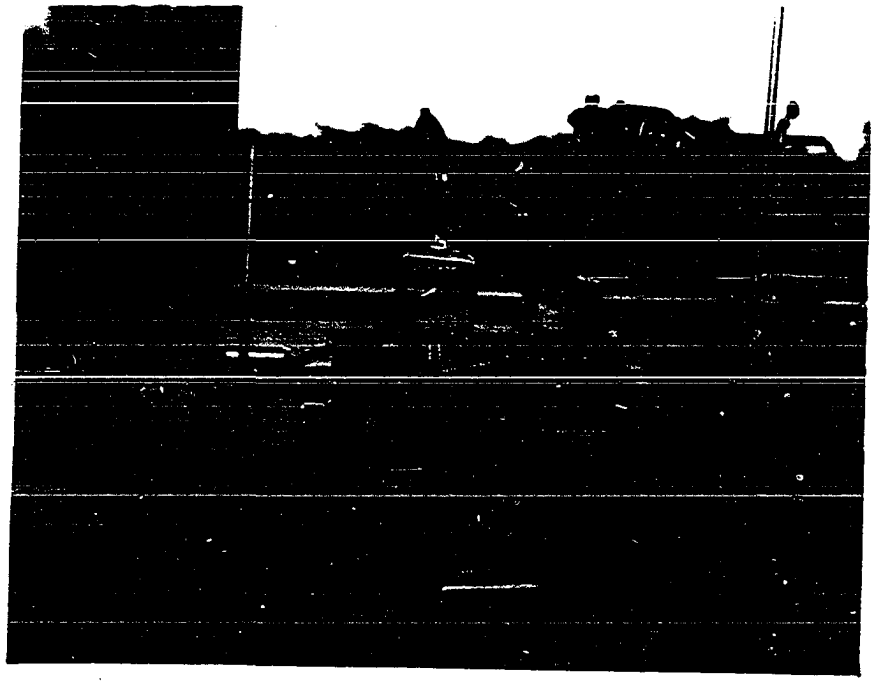
[c]Added in 95 percent ethanol solution

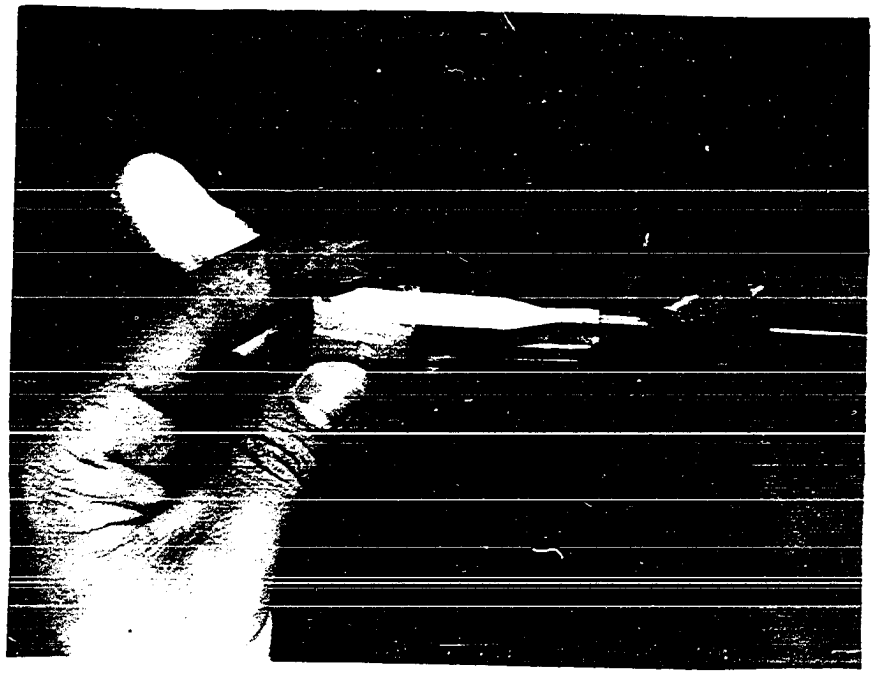
introduced into the bottle to increase the flow rate of the fluid diet. A small pinch clamp placed near the unattached end of the rubber tubing was used to control diet dispensation. The equipment used to dispense the diet is shown in Figure 1. After the diet was poured into the vials, the vials were plugged with cotton and the diet allowed to cool and harden. In order to facilitate initial feeding by the borer, a channel was made down

one side of the media with a small spatula. This procedure is illustrated in Figure 2.

Figure 1. Apparatus used in mixing and pouring the diet

Figure 2. Making a channel down one side of the medium in order to facilitate feeding by the young borer





PROCEDURES AND RESULTS

Microorganism Inhibitors

All existing procedures for rearing the European corn borer on synthetic diets involved the use of cumbersome aseptic handling techniques. Therefore, the first problem considered in the development or modification of a diet that would be satisfactory for the efficient production of a large number of borers was that of developing a medium that could be handled without following aseptic procedures. Earle et al. (1959) accomplished this by the addition of sorbic acid and methyl p-hydroxybenzoate to the diet in the rearing of the boll weevil. Two microorganism inhibitors, sorbic acid and n-butyl p-hydroxybenzoate, were investigated for possible use in a corn borer diet. The levels tested of each of these compounds, individually and in all combinations were: sorbic acid at 0, 0.5, 1.0, and 1.5 percent and Butoben at 0, 0.2, 0.4, and 0.6 percent of the wet weight of the diet. The treatments were arranged in a factorial design with 10 replications. The number of borers in a single treatment in each replication ranged from 2 to 4 with a total of 25 borers being used. The evaluation was started by placing corn borer eggs on the medium being tested. The eggs placed on the control diets were handled in an aseptic manner similar to the one discussed by George (1957). All of the larvae were weighed individually on the tenth day after being placed on the diet, returned to the vials, and allowed to pupate. The criteria used to determine treatment effect in this experiment were larval weight, length of larval period, percent pupation, percent of the young larvae failing to survive after hatching, and the percent of the vials of media which

became contaminated. A summary of the results of this experiment is given in Table 2. A review of the data in Table 2 shows that the media containing the lower concentration of either mold inhibitor in the absence of the other, supported seemingly normal growth of the borer, but both media were heavily contaminated. The combinations of microorganism inhibitors at the lower levels eliminated all visible contamination, and did not seem to greatly affect the growth of the borer. It was concluded that "Butoben" at 0.2 percent and sorbic acid at 0.5 percent of the wet weight of the diet could be used as a microorganism inhibitor mixture in future diets. Extensive use of this combination of microorganism inhibitors in subsequent rearings proved this conclusion to be valid.

Evaluation of Plant Fractions

The second aspect of the problem investigated was the green leaf additive. An additive that would support growth and reproduction at a level similar to that obtained in the field, would enhance rearing programs. Since the corn plant is the major host of the corn borer it was thoroughly tested as a source of the green leaf additive. Tests were designed to determine if any growth response differences could be obtained from the use of different corn plant fractions as the leaf factor in the modified diet. The following fractions were tested for growth and reproduction responses: leaf, whorl, whole plant, and tassel from the susceptible inbred WF9, popcorn tassel and sweet corn kernel. All fractions of WF9, other than tassel, were harvested when the plant reached an extended height of about 40 inches. All of the tassels were harvested shortly before pollination and the kernels were harvested in the soft-

Table 2. Summary of the effect of two mold inhibitors on corn borer larval development

Treatment and concentration		Mean larval weight at 10 days	Length of larval period	Pupation	Contaminated	Failed to survive
		mg	Days	Percent	Percent	Percent
Butoben[a]	0[b]					
Sorbic acid	0	49.6	18.0	66.6	0	0
Butoben	0					
Sorbic acid	0.5	44.6	18.0	5.8[c]	70	0
Butoben	0					
Sorbic acid	1.0	29.0	20.0	74.0	0	0
Butoben	0					
Sorbic acid	1.5	25.1	21.2	83.0	0	0
Butoben	0.2					
Sorbic acid	0	44.9	17.9	90.5	24	0
Butoben	0.2					
Sorbic acid	0.5	40.2	18.8	89.0	0	4
Butoben	0.2					
Sorbic acid	1.0	20.0	20.7	83.0	0	0
Butoben	0.2					
Sorbic acid	1.5	19.6	21.6	88.0	0	4
Butoben	0.4					
Sorbic acid	0	34.5	19.2	82.0	0	0
Butoben	0.4					
Sorbic acid	0.5	26.1	21.2	77.0	0	12
Butoben	0.4					
Sorbic acid	1.0	13.5	23.0	60.0	0	13
Butoben	0.4					
Sorbic acid	1.5	9.4	23.4	87.0	0	18
Butoben	0.6					
Sorbic acid	0	16.8	21.2	71.0	0	27
Butoben	0.6					
Sorbic acid	0.5	16.6	22.3	50.0	0	22
Butoben	0.6					
Sorbic acid	1.0	6.4	26.0	33.0	0	38
Butoben	0.6					
Sorbic acid	1.5	10.0	25.0	50.0	0	16

[a] n-Butyl p-hydroxybenzoate

[b] Percent of the wet weight of the diet

[c] Low figure probably due to very high percent contamination

dough stage. After harvesting, all plant parts were placed on a drying rack made of poultry wire and air dried. They were then ground in a Wiley mill through a 2 mm mesh perforated screen. After grinding, they were placed in a forced ventilation hot air drying oven at 100° F. and left for 72 hours. They were ground again in the Wiley mill, this time using a 0.5 mm mesh perforated screen. After the final grinding, they were stored in a freezer at -4° F. until used. In the first studies, fractions were tested in concentrations of 8, 15, and 26 percent of the dry weight of the diet. It was learned that best response was obtained on the diets containing the highest concentration of plant tissue. Therefore, in the later studies involving the use of soft-dough stage sweet corn kernels and popcorn tassel, only the highest concentration (26 percent) was used. The results of the first tests are illustrated in Figure 3. As can be seen from Figure 3, all tissues other than tassel gave the best results at a dietary level of 26 percent of the dry weight of the diet. A summary of the results obtained from all plant fractions tested at the level of 26 percent is given in Table 3. Also a summary of all the data obtained from the plant fraction evaluation tests is given in Table 14. As can be seen from Table 3, when all three criteria are examined, leaf and whorl gave the best response of any of the tissues tested. In interpreting the results of the experiments using oviposition as the criterion, it is recognized that the population densities and sex ratios within the cages were not identical. The exact effect these variations had on oviposition is not known; therefore, these figures should be viewed with some reservation.

Figure 3. Effects of different corn plant fractions at differing concentrations on larval weight at 10 days

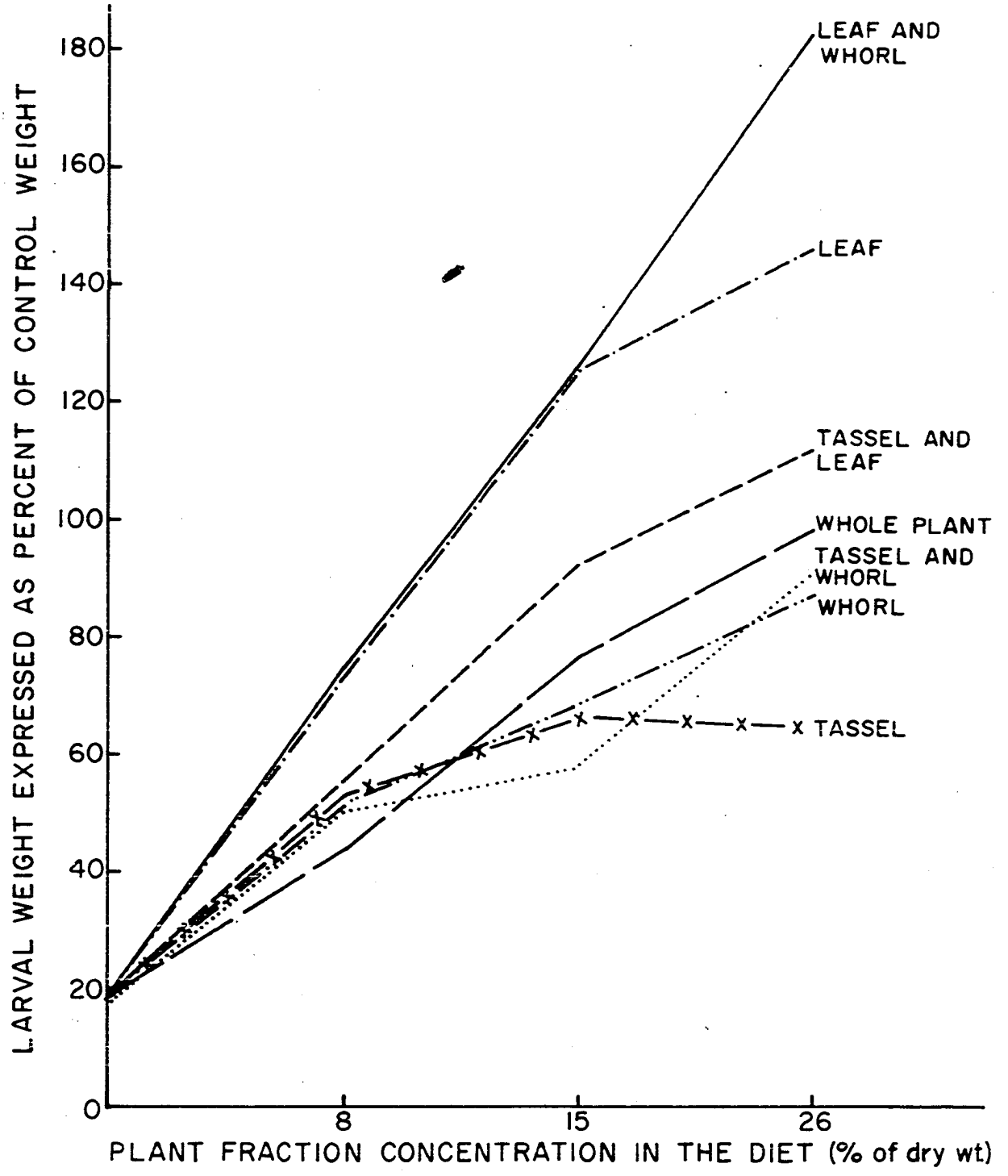

LARVAL WEIGHT EXPRESSED AS PERCENT OF CONTROL WEIGHT
180
160
140
120
100
80
60
40
20
0
8
15
26
PLANT FRACTION CONCENTRATION IN THE DIET (% of dry wt)
LEAF AND WHORL
LEAF
TASSEL AND LEAF
WHOLE PLANT
TASSEL AND WHORL
WHORL
x TASSEL

Table 3. Summary of the responses received from the addition of different plant fractions to the diet

	Larval weight	No. of larvae weighed	Length of larval period	Emergence	No. moths ♀	No. moths ♂	Total No. egg masses	Masses per female
	mg		Days	Percent				
Alfalfa[a]	49.9	19	14.5	68.6	9	15	55	6.1
Leaf[b]	73.4	18	14.1	97.4	21	17	155	7.4
Whorl	43.7	19	14.7	67.5	8	19	17	2.1
Tassel	32.4	18	18.4	96.0	-	-	-	-
Whole plant	49.0	21	14.9	85.3	17	12	36	2.1
Leaf & whorl[c]	90.8	16	15.9	100.0	14	16	176	12.6
Leaf & tassel	55.4	16	16.4	93.0	-	-	-	-
Whorl & tassel	44.4	18	16.9	81.0	-	-	-	-
Corn meal[d]	30.2	19	18.8	17.2	0	5	0	0
Tassel[e]	38.5	25	19.6	53.4	14	6	49	3.5
Tassel[e] & leaf	63.5	25	16.7	83.0	19	15	280	14.7
Tassel[e] & whorl	35.9	25	18.4	45.0	2	17	3	1.5

[a]All fractions were added at the rate of 13.8 grams per 300 grams of diet

[b]All corn tissues are WF9 unless otherwise noted

[c]Combination tissue diets were mixed at the rate of 13.8 grams total with each tissue making up one-half of the total

[d]Sweet corn kernel

[e]Popcorn tassel

Another difficulty encountered in interpreting the results from this experiment and all of the other experiments based on growth rate of the larvae is the large variability in larval weight. This variation arises from the presence of larvae that fail to develop properly and remain small. Another problem encountered is the fact that the number of larvae surviving to 10 days of age is very seldom the same on each treatment. This factor of unequal numbers of surviving larvae and the failure of others to develop normally results in data that are difficult to handle statistically. This is why the data were not evaluated statistically even though the experiments were planned and initiated in such a manner that they could be statistically evaluated. Table 15 has been included to illustrate the variability mentioned above.

An interesting sidelight of the results of this experiment is the fact that larvae of the first brood concentrate and feed in the whorl of the corn plant. This experiment showed that of all plant parts tested, the whorl additive resulted in the poorest response, however, when used in combination with the leaf portion it gave a response greater than leaf alone. When egg masses per female was used as the major criterion, the leaf plus tassel (WF9 leaf and popcorn tassel) gave the best response. Since leaf and whorl gave the best over-all results, it was decided to use this combination for the experiments on continuous rearing.

Continuous Rearing

The introduction of corn plant fractions composed of mixtures of leaves and whorl and the successful use of the microorganism inhibitors, sorbic acid and butoben, resulted in a diet that appeared to be reason-

ably satisfactory for the rearing of the corn borer in the laboratory. However, to test the adequacy of the diet, a program of continuous rearing was initiated.

The method of starting the borers on the diet varied as the work progressed. In earlier work individual eggs were placed in the vials. In later generations egg masses were allowed to hatch in a moist container and the young larvae were picked up on a narrow spatula and placed in the vials, two to a vial. This removed the variable of egg hatchability from the results of the experiment. Records were taken on days to first pupation and failure to pupate in 20 to 21 days. Pupae were placed in sterile cages for emergence of moths and egg laying. After records of the number of egg masses laid had been completed and all moths in the cage were dead, the cages were opened. The number of males and females was determined and pupal mortality noted. The results of the continuous rearing experiments are given in Table 4. Egg masses per female was used as the major index to borer vitality in this experiment. From the data given in Table 4, it is evident that results from generation to generation were variable. No loss in vitality is indicated by these data after 13 generations of rearing. Figure 4 compares 13th generation laboratory-reared moths with moths which are the progeny of field-collected larvae.

Chemical Definition of the Diet

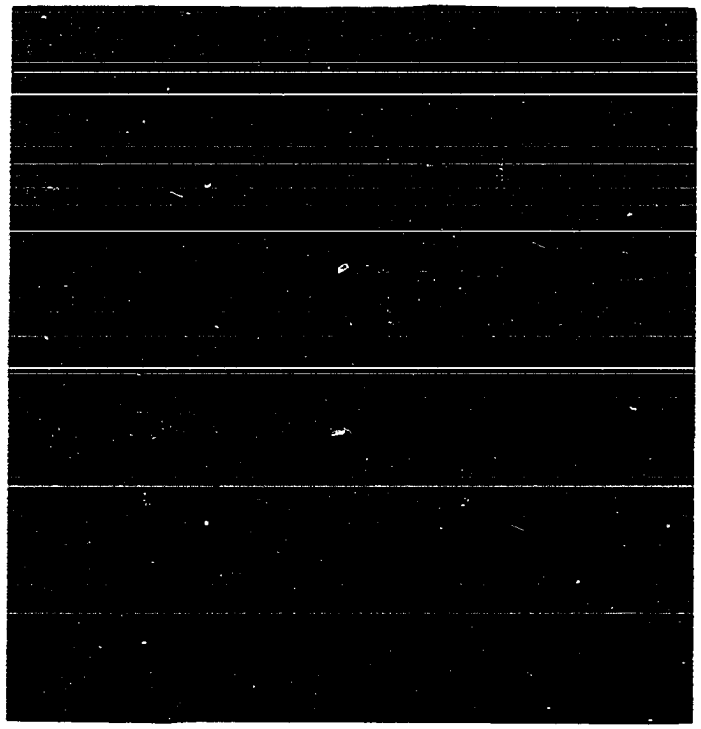
The diet changes, discussed in the preceding sections, resulted in a diet that was remarkably satisfactory for quantity production of corn borers for laboratory uses. However, three ingredients in this growth medium, namely, brewer's yeast, casein, and corn leaf, contain many

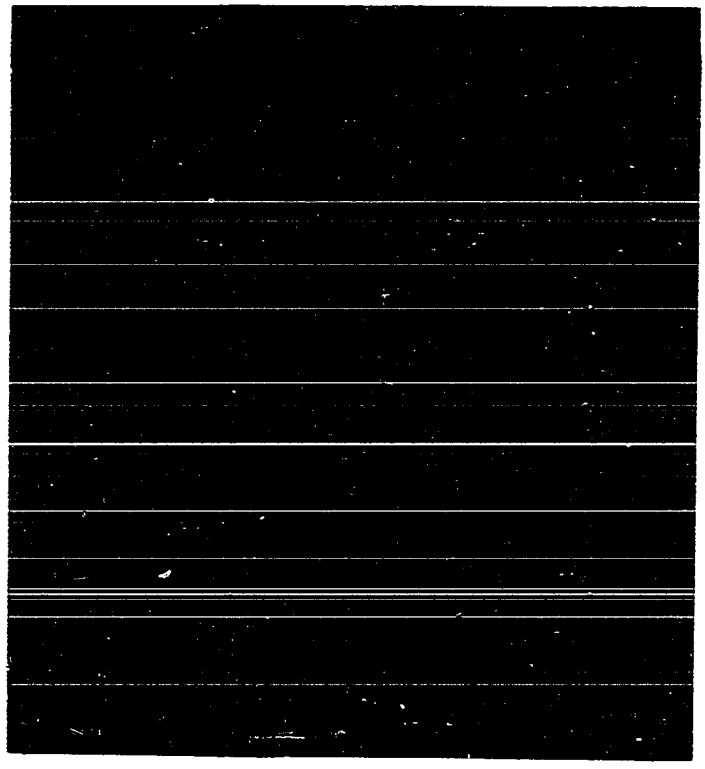
Table 4. Summary of data on continuous rearing of European corn borer on artificial media

Generation	Larvae at 10 days	Pupation		Emergence		Egg masses per female
	Number	Number	Percent	Number	Percent	Number
			Culture 1			
I	215	173	80.4	133	76.8	11.8
II	331	275	83.0	199	72.3	9.1
III	457	347	75.9	242	69.7	7.4
IV	763	641	84.0	443	69.1	8.3
V	465	402	86.5	261	64.9	11.6
VI	336	319	94.9	208	65.2	10.1
VII	308	290	94.2	212	73.0	8.4
VIII	318	281	88.3	197	70.0	8.3
			Culture 2			
I	-	157	-	110	70.1	10.4
II	159	120	75.5	98	81.7	19.1
III	179	159	88.8	126	79.2	17.0
IV	230	177	77.0	102	57.6	5.1
V	265	199	75.1	139	69.8	6.9
VI	327	280	85.6	207	73.9	8.2
VII	270	242	89.6	168	69.4	10.6
VIII	264	230	87.1	177	77.0	4.9
IX	243	217	89.3	158	72.8	6.9
X	313	281	89.7	180	64.0	9.3
XI	156	115	74.1	90	78.3	7.1
XII	258	217	84.1	177	81.5	3.9
XIII	193	161	83.4	124	77.0	10.1

compounds whose function in the nutrition of the borer is unknown. The replacement of these products with chemically defined materials would make the diet more satisfactory for the evaluation of exact dietary requirements of the corn borer. Chemical definition of the unknown components of the medium also would make the medium a more useful tool in the bioassays

Figure 4. The moths at the top are from field-collected pupae, and the ones at the bottom are from the 13th generation reared on artificial media





of corn inbreds and hybrids to determine if they contain chemicals which are responsible for the resistance of some corns to the development of the borer, or to determine the presence of growth factors that enhance corn borer vitality. A series of experiments was designed to further characterize some of these ingredients.

Corn leaf factor characterization

Several compounds have been checked for corn leaf factor activity. Beck (1953) tested thiamine, riboflavin, pyridoxine, pantothenic acid, nicotinic acid, choline, inositol, para-aminobenzoic acid, biotin, folic acid, sodium nucleate, adinine, ascorbic acid, and carnitine for corn leaf activity. All indicated negative results. In this investigation the first materials tested were glutathione and the 18 natural L-amino acids. The amino acids that were tested are listed in Table 9. The amino acids were not tested individually, but as a mixture. The tests of the glutathione and amino acids produced negative results. Since this procedure failed, a series of experiments was designed to study the corn leaf factor by chemical fractionation.

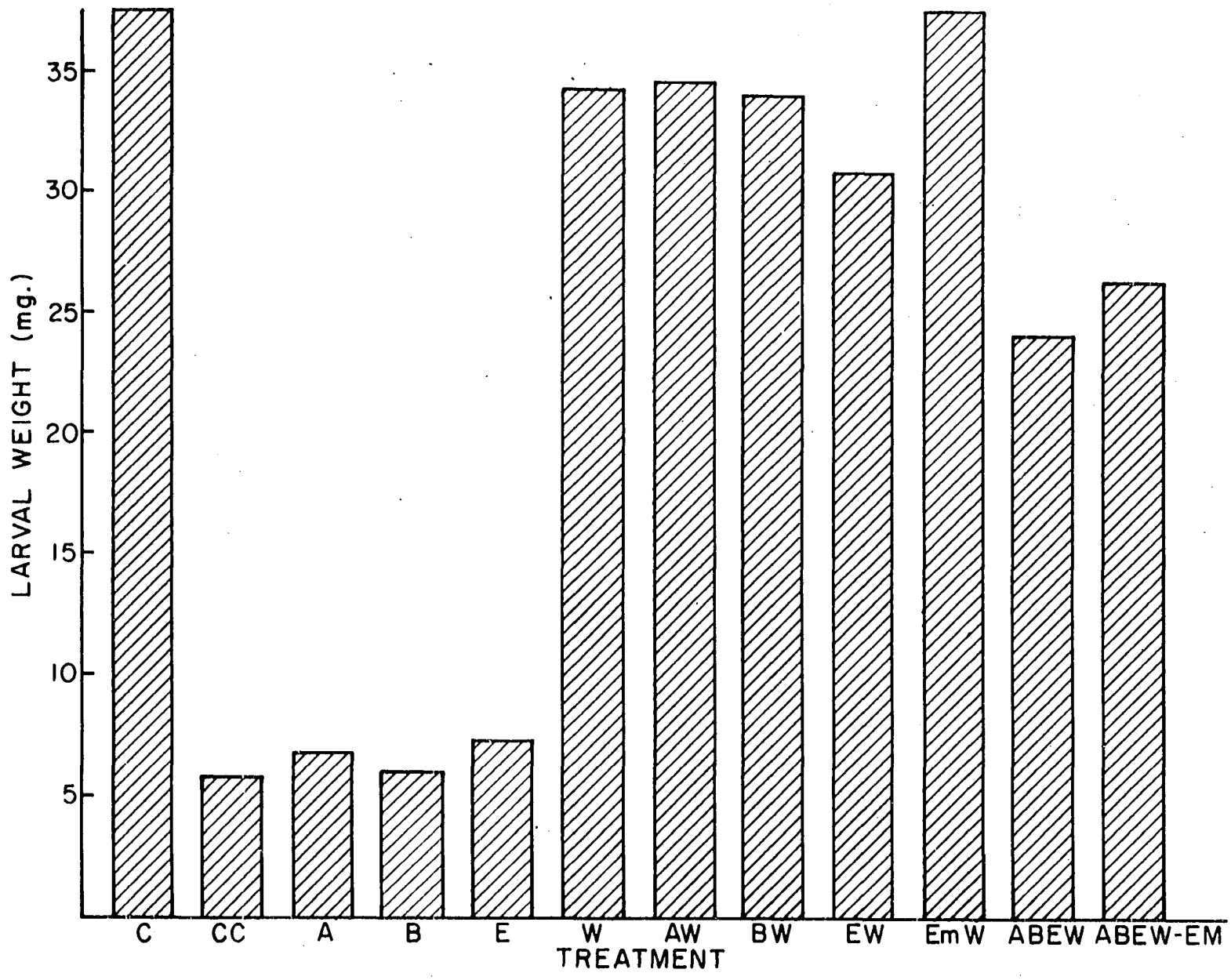
Solvent extraction It was felt that the solubility characteristics of the corn leaf factor should be the first property determined. Therefore, dried corn leaf material was extracted in a Soxhlet extractor with four solvents in the following order: alcohol, ether, benzene, and water. With this extraction apparatus it was possible to extract only 91 percent of the leaf factor. Therefore, 15 grams of leaf was extracted to obtain the same activity in the extract as was obtained in 13.8 grams of dried corn leaf. The leaf was extracted with each solvent for 72 hours.

In the case of the toxic solvents, alcohol, ether, and benzene, the solvent containing the leaf material which was soluble in it was poured on 4 grams of cellulose and then the solvent was removed in a rotary evaporator under reduced pressure. The cellulose with the extract associated with it was added to the diet. In the case of the water extract, pure cellulose was substituted for the corn leaf and the water extract was added in lieu of part of the distilled water. Each of the fractions was bioassayed using a diet containing dried corn leaf as the check. Each individual extract, all combinations of the other extracts with the water extract, and the combination consisting of all four solvent extracts were tested for biological activity. The results of these assays are presented in Figure 5. A summary of the data obtained from these experiments is given in Table 16. As can be seen from Figure 5, the water soluble fraction of the corn leaf was the only fraction tested that was biologically active. The combination of the other fractions with the water soluble fraction did not result in an increase in response above that received from the water extract alone nor did the combination of all fractions.

Dialysis Following the determination that the growth factor appeared to be water soluble, experiments were conducted to determine if it was dialyzable. The aqueous extract from 15 grams of corn leaf was placed in solution in 30 ml of glass distilled water and the resulting mixture placed in commercially obtained dialyzing tubing. The tubing was tied at both ends and emersed in 100 ml of glass distilled water. The dialyzing water was changed every 8 to 10 hours for 72 hours. After this

Figure 5. Results of the bioassay of the four solvent fractions of the corn leaf

C - Control
CC - Cellulose added for the corn leaf
A - Alcohol soluble fraction
B - Benzene soluble fraction
E - Ether soluble fraction
W - Water soluble fraction
AW - Combination of alcohol and water soluble fraction
BW - Combination of benzene and water soluble fraction
EW - Combination of ether and water soluble fraction
EM-W - Combination of the leaf material after the extracts have been made from it and the water soluble fraction
ABEW - Combination of all of the solvent soluble fractions
ABEW-EM - Combination of all of the solvent soluble fractions and the extracted leaf material


LARVAL WEIGHT (mg.)
35
30
25
20
15
10
5
C
CC
A
B
E
W
AW
BW
EW
EmW
ABEW
ABEW-EM
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time period all of the dialyzing water used was condensed to 30 ml in a rotary evaporator. The dialyzable and the non-dialyzable portions were assayed for biological activity. The results of the assays are summarized in Table 5. It is strongly indicated in this experiment that the corn leaf factor is dialyzable. This agrees with the conclusion of Beck (1953).

Table 5. Effects of dialysis on the biological activity of the water soluble portion of the corn leaf

Treatment	Larvae	Mean larval weight	Length of larval period	Pupation	Emergence
	No.	mg	Days	Percent	Percent
Control[a]	46	38.0	17.4	100	91
ND[b]	36	17.4	21.6	100	87
D[c]	43	38.4	17.1	100	53

[a]Unmodified water extract of leaf material and cellulose substituted for the leaf material in the basic diet

[b]Non-dialyzable portion of the water extract

[c]Dialyzable portion of the water extract

Acid hydrolysis The dialyzable portion of the water soluble material from 15 grams of corn leaf was put into solution in 30 ml of distilled water. Thirty milliliters of concentrated hydrochloric acid was added to the solution and the resulting mixture was refluxed for 7 hours. After refluxing the resulting mixture was evaporated to dryness. Then 60 ml of distilled water was added to the dried material. This procedure of adding distilled water and evaporating to dryness was repeated three

times and then the pH of the final aqueous mixture was adjusted back to the pH of the crude leaf extract with NaOH. This mixture was added to the diet and bioassayed. In order to check on the possibility of a detrimental effect on borer growth by salt which might have been produced in the process, a salt check diet was devised. The amount of the dilute solution of sodium hydroxide required to adjust the pH of the hydrolyzed leaf material back to the pH of the crude leaf extract was adjusted to the pH of the crude leaf extract by the addition of dilute hydrochloric acid. This mixture of hydrochloric acid and sodium hydroxide was added to one control diet to serve as the salt check. The results of the acid hydrolysis test are given in Table 6. The results of this experiment show that the biological activity of the corn leaf factor is destroyed by acid hydrolysis. This is not in agreement with Beck's (1953) findings. However, the methods used by him were not described so a direct comparison was not possible.

Table 6. Effects of acid hydrolysis on the biological activity of the corn leaf

Treatment	Larvae	Mean larval weight	Length of larval period	Pupation	Emergence
	No.	mg	Days	Percent	Percent
Control	44	38.8	20.2	91	87
Acid Hydrolyzed	0	4.8	-	0	0
Salt Check	41	31.0	18.7	100	89

Ion exchange chromotography The next procedure to further characterize the corn leaf factor was to attempt to partition the factor or to separate it from other factors in the leaf extract by the use of ion

exchange chromotography. Three ion exchange resins were used: Amberlite IR-45, an OH^- type anion resin, Amberlite IRC-50, a H^+ type cation resin and Amberlite MB-3, a mixed bed resin. The columns used were 3 cm in diameter and 39 cm in length. The columns were packed by making a slurry mixture of deionized glass distilled water and the ion exchange resin and pouring this mixture into the top of the columns. The resin was allowed to settle and then the excess water was removed until approximately 0.5 inch of water remained on top of the resin. Thirty milliliters of water containing the dialyzable water soluble extract of 15 grams of corn leaf were added slowly to the top of the column and allowed to pass through the column at the rate of 2 ml per minute. When all of the extract had been added to the column and approximately 0.5 inch of it remained above the top of the resin, 1.5 column volumes of deionized glass distilled water were passed through the column. All of the material coming through the column was then assayed for biological activity. The results of these assays are given in Table 7. It is indicated in these tests that if the corn leaf factor is charged, it is only weakly charged.

Removal of brewer's yeast

Since it had already been determined that the corn leaf factor required in the diet was water soluble and dialyzable, a dialyzed water extract of the leaf material was incorporated into the diet as the leaf factor in all subsequent feeding trials. Also, cellulose was added in all diets in place of the constituent removed in an attempt to retain approximately the same texture in the test diets as in the basal diets. From

Table 7. The biological activity of the corn leaf factor after passing the water extract through ion exchange resins

Treatment	Larvae	Mean larval weight	Length of larval period	Pupation
	No.	mg	Days	Percent
Control[a]	43	30.6	19.1	100
A.E.[b]	44	21.7	19.9	96
C.E.[c]	38	14.6	21.8	97
MB.E.[d]	42	13.4	25.6	90

[a]Dialyzable portion of the water extract and cellulose substituted for the leaf material in the basic diet

[b]Dialyzable portion of the water extract passed through an anion exchange resin

[c]Dialyzable portion of the water extract passed through a cation exchange resin

[d]Dialyzable portion of the water extract passed through a mixed bed exchange resin

information received from Mr. S. M. Mann*, it was known that yeast is high in the major B-vitamins, therefore, they were first added in the place of the brewer's yeast. The B-vitamins used are given in Table 8. These vitamins were added at the rate of 0.5, 1, 2, and 4 ml per 300-gram diet. The results of the first feeding trial are presented graphically in Figure 6. A summary of all data obtained from the first tests is given in Table 17. It is apparent, as shown in Figure 6, that the B-vitamin mixture resulted in the greatest response when it was included

*Mann, S. M., Nutritional Biochemicals Corporation, 21010 Miles Avenue, Cleveland 28, Ohio. Analysis of brewer's yeast. Private communication. 1960.

Table 8. Major B-vitamins used in brewer's yeast replacement tests

Vitamin	Amount contained in 1 cc
	mg
Thiamine HCl	5.0
Riboflavin	2.0
Nicotiamide	75.0
Pantothenic Acid	2.5
Pyridoxine HCl	5.0
Vitamin B_{12}	2.5

at the rate of 0.5 ml per 300-gram diet.

In the second feeding trial 0.225 mg of folic acid, 0.09 mg of biotin, 90 mg of inositol and an additional 2 grams of glucose were added in addition to 0.25 ml of the B-vitamin mixture. The results of the second feeding trial are shown in Figure 7. A further summary of all data obtained from this series of feeding trials is given in Table 18. From Figure 7 it is evident that brewer's yeast can be replaced in the diet by 0.25 ml of the B-vitamin mixture, 90 mg of inositol, 0.225 mg of folic acid, 0.09 mg biotin and 2 grams of glucose, without any apparent effect on borer growth. However, with the exception of glucose, none of the above components were tested individually to determine the exact requirements of each.

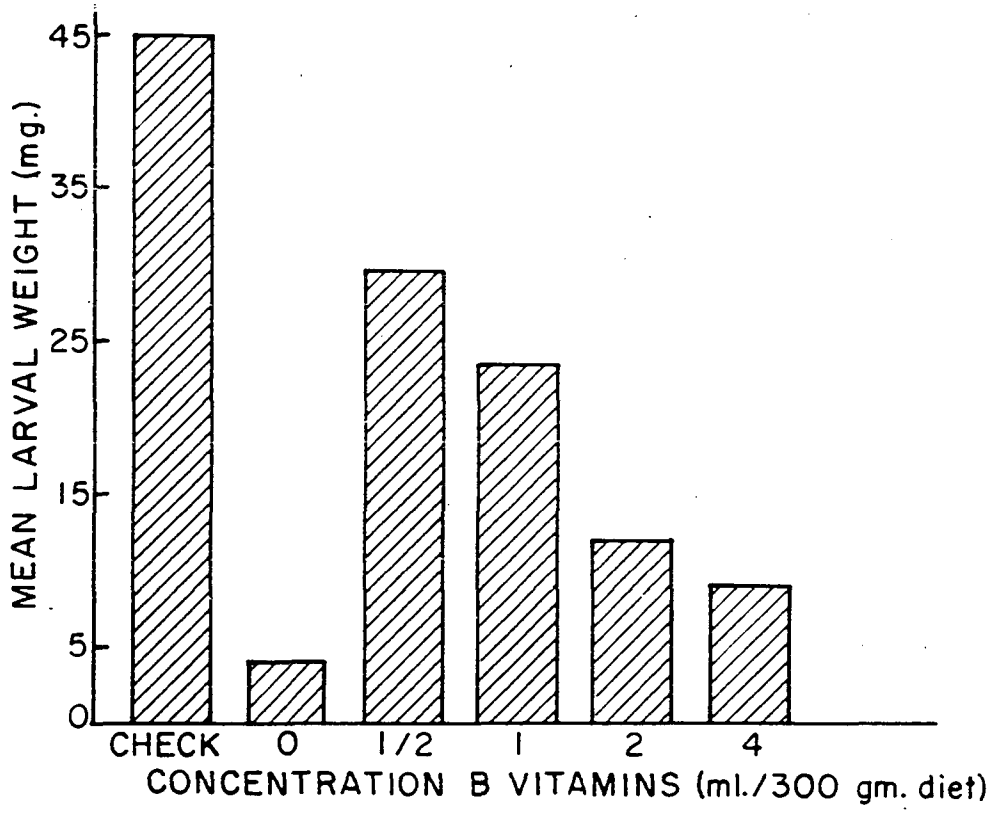
Removal of casein

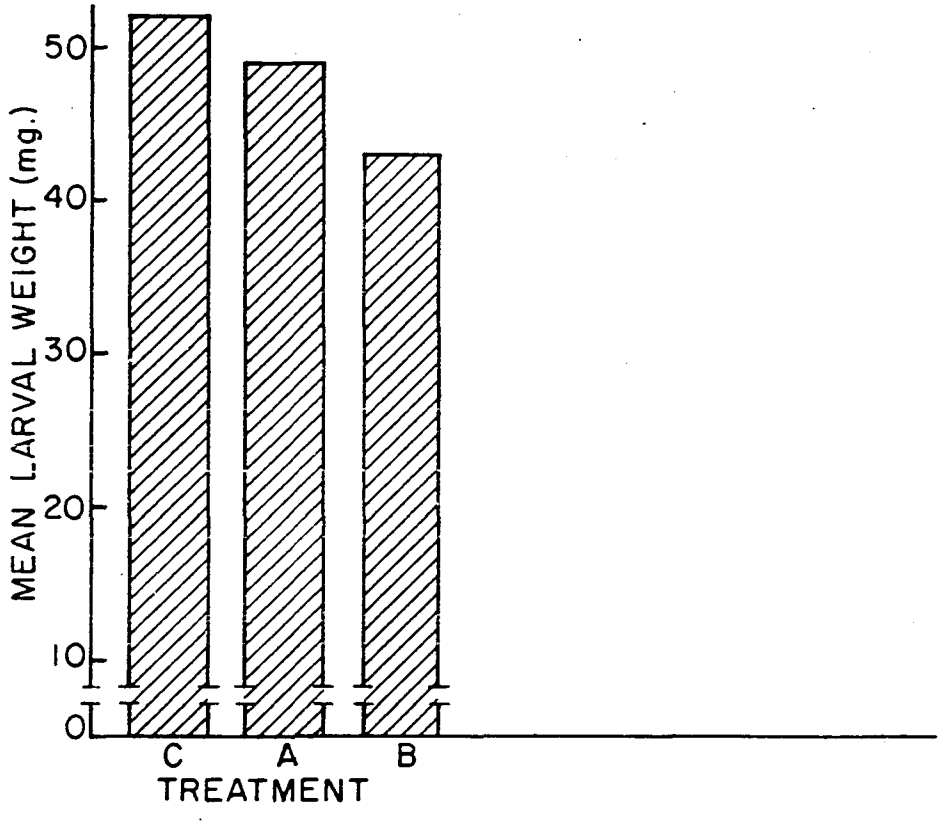
The next attempt in further defining the diet was the removal of casein. A typical analysis of casein showed that it was considerably high in amino acids. Therefore a mixture of 18 of the natural-L-amino acids was substituted for casein in the diet. The amino acids and amounts of amino acids included were based upon the amino acid composition of

Figure 6. Effect of substituting various concentrations of the major B-vitamins for brewer's yeast powder in the basal diet

Figure 7. Effect of substituting two concentrations of the B-vitamins, inositol, folic acid, biotin, and glucose for brewer's yeast powder in the basal diet

C - Control
A - 0.25 ml of the B-vitamin mixture, 90 mg of inositol, 0.225 mg of folic acid, 0.09 mg biotin, and an additional 2 grams of glucose per 300 grams of basil diet
B - Same as A except 0.5 ml of B-vitamin mixture


MEAN LARVAL WEIGHT (mg.)
45
35
25
15
5
0
CHECK
0
1/2
1
2
4
CONCENTRATION B VITAMINS (ml./300 gm. diet)


MEAN LARVAL WEIGHT (mg.)
50
40
30
20
10
0
C
A
B
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casein as indicated by Mr. S. M. Mann*. These are listed in Table 9.

The results of substituting the amino acid mixture in Table 9 for casein, are given in Table 10. These data show that casein can be eliminated from the diet by adding a mixture of L-amino acids.

Table 9. Amino acids and amount of each used in the casein substitution experiment

Amino Acid	Amount added to each 300-gram diet	Amino Acid	Amount added to each 300-gram diet
	mg		mg
Alanine	200	Lysine	230
Arginine	160	Methionine	70
Aspartic Acid	400	Phenylalanine	200
Cystine	60	Proline	270
Glutamic Acid	450	Threonine	170
Glycine	130	Tryptophan	60
Histidine	110	Tyrosine	130
Iso-Leucine	220	Serine	450
Leucine	680	Valine	230

Table 10. Effects of substituting an amino acid mixture for casein

	Larvae	Mean larval weight	Length of larval period	Pupation	Emergence
	No.	mg	Days	Percent	Percent
Control	47	46.0	17.3	96	85
0	42	12.0	20.9	90	95
Amino Acids	40	40.4	18.8	95	95

*Mann, S. M., Nutritional Biochemicals Corporation, 21010 Miles Avenue, Cleveland 28, Ohio. Analysis of casein. Private communication. 1960.

Removal of casein and brewer's yeast simultaneously

After obtaining compounds that would replace the casein and brewer's yeast in the diet, the next step was to remove both from the diet at the same time. It was already established that brewer's yeast could be removed from the diet by adding 0.25 ml of the B-vitamin mixture given in Table 8, 90 mg of inositol, 0.225 mg of folic acid, 0.09 mg biotin and 2 grams of glucose, and that casein could be replaced in the diet by a mixture of the 18 L-amino acids listed in Table 9. Therefore an experiment was devised and carried out to replace both brewer's yeast and casein in the diet. Information received from Mr. S. M. Mann* indicated that brewer's yeast contains some amino acids. Therefore, the amino acid composition used in the casein replacement trials was altered for use in this experiment. The amino acid mixture used in this experiment is listed in Table 11. Six combinations of three different concentrations of the

Table 11. Amino acid mixture used in casein and brewer's yeast replacement tests

Amino Acid	Amount	Amino Acid	Amount
	grams		grams
Arginine	0.123	Valine	0.173
Histidine	0.081	Alanine	0.062
Isoleucine	0.173	Asparatic acid	0.132
Leucine	0.229	Glutamic acid	0.143
Lysine	0.171	Glycine	0.041
Methionine	0.036	Proline	0.084
Phenylalanine	0.151	Serine	0.146
Threonine	0.132	Cystine	0.017
Tryptophan	0.045	Tyrosine	0.040

*Mann, S. M. Nutritional Biochemicals Corporation, 21010 Miles Avenue, Cleveland 28, Ohio. Analysis of brewer's yeast. Private communication. 1960.

amino acid mixture and two different concentrations of glucose were tested. The composition and concentration of the dietary components used in the brewer's yeast-casein replacement trials are given in Table 12. The results from the replacement of brewer's yeast and casein with the mixtures described in Table 12 are given in Table 13. If mean larval

Table 12. Composition and concentration of the components used to replace brewer's yeast and casein in the basal diet

Dietary component		Test mixture number					
		I	II	III	IV	V	VI
B-vitamin mixture	(ml)	0.25	0.25	0.25	0.25	0.25	0.25
Inositol	(mg)	90.0	90.0	90.0	90.0	90.0	90.0
Folic Acid	(mg)	0.225	0.225	0.225	0.225	0.225	0.225
Biotin	(mg)	0.09	0.09	0.09	0.09	0.09	0.09
Amino Acid mixture	(gm)	2.968	5.937	8.905	2.968	5.937	8.905
Glucose[a]	(gm)	13.0	13.0	13.0	15.0	15.0	15.0
Cellulose[b]	(gm)	18.0	18.0	18.0	18.0	18.0	18.0

[a]Basic diet contained 10.5 grams glucose

[b]Control diet with water extract of leaf as the leaf factor additive had 10 grams of cellulose

Table 13. Summary of results of the experimentation on the simultaneous replacement of brewer's yeast and casein

Diet number	Larvae	Mean larval weight	Average length of larval period	Pupation	Emergence
	No.	mg	Days	Percent	Percent
Control	32	70.4	15.3	100	100
I	36	3.5	-	0	0
II	42	12.6	21.4	86	89
III	38	19.7	20.6	88	86
IV	46	4.2	25.4	28	100
V	44	8.4	23.1	90	84
VI	38	23.8	17.8	94	94

weight at 10 days is used as the major criterion, then these results clearly indicate that none of the diets tested contained the proper ingredients or the proper concentration of ingredients to replace both casein and brewer's yeast in the basic diet. However, results based on the other criteria indicate that Diet No. VI is adequate for rearing the borer to maturity and allowing it to complete the developmental processes. This diet is sufficient for limited use in further clarification of the nutritional requirements of the insect. However, more work should be done with altering the concentrations of ingredients in this diet.

CONCLUSIONS

The European corn borer can be reared on the meridic diet given in this thesis for an indefinite period of time without any apparent loss of vitality.

The addition of microorganism inhibitors to this diet did not have any appreciable effect on borer growth and allows the borers and diet to be handled without following aseptic procedures. The elimination of aseptic handling procedures more than triples the number of borers that may be started on the media in any given period of time.

The green leaf factor required in the meridic diet is water soluble, heat stable up to temperatures of 100° C., dialyzable, acid hydrolyzable and weakly charged.

Brewer's yeast may be replaced in this diet with a mixture of vitamins and additional glucose without any effect on larval growth or borer development.

Casein can be replaced in the diet with 18 of the natural L-amino acids without any apparent adverse effect on the borers.

No combination of compounds was found that would replace both casein and brewer's yeast without a detrimental effect on larval growth rate. However, the replacement of these two constituents with a mixture of vitamins, additional glucose, cellulose, and a mixture of 18 of the natural L-amino acids did not have an adverse effect on pupation or emergence.

SUMMARY

The objective of this study was to develop a medium that would be satisfactory for rearing large numbers of European corn borers. Three major areas were studied: (1) the modification of existing diets for adaptation in a mass rearing program, (2) the development and reproduction responses of the borers after being reared continually on a synthetic diet for several generations, and (3) the development of a more precisely defined diet.

The use of aseptic procedures was one of the limiting factors in developing an efficient method of rearing large numbers of borers. This study demonstrated that the addition of sorbic acid at 0.5 percent of the weight of the diet and n-butyl p-hydroxybenzoate at 0.2 percent of the diet to the growth media controlled all visible contamination without greatly affecting the growth rate of the borer. Elimination of the aseptic handling procedures greatly reduced the time required to process diets and initiate the rearing procedures.

A satisfactory source of the green leaf additive was found to be a mixture of WF9 leaf and whorl at a level of 26 percent of the dry weight of the diet. This addition resulted in a medium on which vigorous borers could be raised.

Thirteen generations of the corn borer were reared on the diet containing microorganism inhibitors and the above-mentioned green leaf additive. The borers reared on this diet did not show any indication of a loss of vitality.

The presence of crude materials of unknown composition in a diet

limits the use of such media in bioassay experiments and in the clarification of the exact nutritional requirements of the corn borer. Therefore, several experiments were carried out in an attempt to develop a more chemically defined diet which would be adequate for the corn borer. These experiments are summarized below.

From several experiments devised and carried out to further characterize the nature of the corn leaf factor, it was found that it is water soluble, heat stable up to temperatures of 100° C., dialyzable, acid hydrolyzable and weakly charged.

Brewer's yeast can be replaced in the basal diet by 1.25 mg thiamine HCl, 0.5 mg of riboflavin, 18.5 mg of nicotinamide, 0.625 mg of pantothenic acid, 1.25 mg of pyridoxine HCl, 0.625 mg of vitamin B_{12}, 90 mg of inositol, 0.225 mg of folic acid, 0.09 mg of biotin, and an additional 2 grams of glucose per 300 grams of diet (wet weight) without any apparent effect on borer growth.

The replacement of casein in the basal diet can be accomplished without great effect on borer growth by the addition of the following amino acids in the amounts indicated: 200 mg of alanine, 160 mg of arginine, 400 mg of aspartic acid, 60 mg of cystine, 450 mg of glutamic acid, 130 mg of glycine, 110 mg of histidine, 220 mg of isoleucine, 680 mg of leucine, 230 mg of lysine, 70 mg of methionine, 200 mg of phenylalanine, 270 mg of proline, 170 mg of threonine, 60 mg of tryptophan, 130 mg of tyrosine, 450 mg of serine, and 230 mg of valine per 300-gram diet (wet weight).

Casein and brewer's yeast cannot be simultaneously replaced in the diet with any of the combinations of materials at the concentrations

tested without measurable effect on borer growth. However, the substitution of 1.25 mg of thiamine HCl, 0.5 mg of riboflavin, 18.5 mg of nicotinamide, 0.625 mg of pantothenic acid, 1.25 mg of pyridoxine HCl, 0.625 mg of vitamin B_{12}, 90 mg of inositol, 0.225 mg of folic acid, 0.09 mg of biotin, and 200 mg of alanine, 160 mg of arginine, 400 mg of aspartic acid, 60 mg of cystine, 450 mg of glutamic acid, 130 mg of glycine, 110 mg of histidine, 220 mg of isoleucine, 680 mg of leucine, 230 mg of lysine, 70 mg of methionine, 200 mg of phenylalanine, 270 mg of proline, 170 mg of threonine, 60 mg of tryptophan, 130 mg of tyrosine, 450 mg of serine, and 230 mg of valine, and an additional 4.5 grams of glucose and 18 grams of cellulose per 300-gram diet for brewer's yeast and casein in the presence of a modified water extract of corn leaf allowed borers to develop to the adult stage.

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APPENDIX

Table 14. Results of WF9 plant fraction evaluations

Tissue and concentration	Mean larval weight	Larvae weighed	Length of larval period	Emergence	Adults ♀	Adults ♂	Masses per female
Percent	mg	No.	Days	Percent	No.	No.	No.
Control	49.8	20	15.1	78.8	10	15	1.0
Leaf							
8	38.2	20	17.3	87.2	14	20	1.6
15	62.0	16	14.9	94.3	11	22	6.7
26	73.2	18	14.7	97.4	21	17	7.4
Whorl							
8	24.3	18	19.2	37.0	1	12	4.0
15	34.0	19	17.0	38.7	3	9	0
26	43.6	19	15.3	67.5	8	19	2.1
Leaf and whorl							
8	37.9	16	16.5	63.3	7	12	3.0
15	62.0	15	15.5	100.0	12	19	2.0
26	90.5	16	14.4	100.0	14	16	9.0
Tassel							
8	27.1	18	-	-	-	-	-
15	33.1	18	-	-	-	-	-
26	32.4	21	-	-	-	-	-
Tassel and leaf							
8	27.4	20	-	-	-	-	-
15	45.5	14	-	-	-	-	-
26	55.5	16	-	-	-	-	-
Tassel and whorl							
8	23.3	19	-	-	-	-	-
15	29.5	18	-	-	-	-	-
26	44.3	18	-	-	-	-	-
Whole plant							
8	21.5	19	19.1	44.8	6	7	0
15	37.7	20	16.9	86.7	9	17	0.9
26		21	15.8	85.3	12	17	4.2

Table 15. Larval weights of borers reared on diets containing leaf and whorl from WF9

Replication	Control	Leaf			Whorl		
		3.45[a]	6.90	13.80	3.45	6.90	13.8
	mg	mg	mg	mg	mg	mg	mg
IV	80.4	26.6	74.8	103.8	33.8	27.6	44.8
	73.4	48.6	44.6	96.6	19.0	26.0	77.4
	34.6	40.4	D[b]	63.8	29.4	32.0	82.6
V	35.8	29.2	77.4	64.2	28.6	28.4	43.0
	83.4	33.4	45.6	125.6	9.8	27.4	28.8
	65.0	34.4	D[b]	85.4	D[b]	28.0	D[b]
VI	21.2	36.0	42.0	40.6	30.2	49.6	45.8
	25.2	48.4	43.6	66.0	18.6	32.0	25.8
	29.4	44.6	61.6	D[b]	30.4	30.4	21.2
	41.6	50.0	44.4	D[b]	15.6	24.8	32.0
VII	59.4	28.0	69.2	67.8	20.6	49.8	39.1
	43.0[b]	30.0	56.6[b]	64.8	25.2	46.2	36.4
	D	39.4	D	60.0	FTH[c]	38.0	21.6
VIII	73.9	49.4	85.4	63.6	31.6	49.4	45.8
	42.0	29.0	88.8	56.2	12.0	25.0	56.8
	27.4	56.0	66.6	79.5	25.4	36.0	21.2
IX	69.8	48.6	62.2	83.4	20.4	33.6	26.2
	34.8	35.6	70.0	67.2	12.4	22.2	59.0
	64.6	29.2	42.0	73.4	38.6	27.4	47.4
	44.4	12.2	D[b]	61.2	27.9	D[b]	75.2
Mean	49.9	37.5	60.9	73.4	63.9	33.4	43.7
No. larvae weighed	19	20	16	18	18	19	19

[a]Grams per 300-gram diet (wet weight)

[b]Larvae dead at time of weighing

[c]Larvae dead at 5 days

Table 16. Summary of the data obtained from the leaf extraction experiments

Treatment	Mean larval weight	Length of larval period	Pupation	Emergence	Egg masses per female
	mg	Days	Percent	Percent	No.
Control[a]	37.4	17.1	96	90	14.2
CC[b]	5.8	24.0	6	50	0
A[c]	6.7	-	0	0	0
B[d]	6.1	25.0	3	0	0
E[e]	7.3	23.0	5	0	0
W[f]	34.0	17.6	92	91	16.8
AW[g]	34.6	17.0	96	83	13.5
BW[h]	34.1	17.1	90	-	-
EW[i]	30.8	17.4	96	-	-
EM-W[j]	38.6	17.0	96	100	12.7
ABEW[k]	23.3	18.5	92	91	13.7
ABEW-EM[l]	24.6	17.7	92	83	8.8

[a]Control: Basic diet

[b]CC: Cellulose added for the corn leaf

[c]A: Alcohol soluble fraction

[d]B: Benzene soluble fraction

[e]E: Ether soluble fraction

[f]W: Water soluble fraction

[g]AW: Combination of alcohol and water soluble fraction

[h]BW: Combination of benzene and water soluble fraction

[i]EW: Combination of ether and water soluble fraction

[j]EM-W: Combination of leaf material after extraction with above four solvents and water soluble fraction

[k]ABEW: Combination of the four solvent soluble fractions

[l]ABEW-EM: Combination of the four solvent soluble fractions and the extracted leaf material

Table 17. Summary of the data obtained from substituting various concentrations of the B-vitamins for brewer's yeast

Treatment	Mean larval weight	Larvae weighed	Length of larval period	Pupation
	mg	No.	Days	Percent
Control[a]	43.3	36	18	100
0	3.7	30	-	0
0.5 ml[b]	28.8	35	18	100
1 ml	13.0	35	25.6	40
2 ml	10.9	37	30	22
4 ml	8.0	35	31	6

[a]Control: Basic diet

[b]Components of B-vitamin mixture: thiamine HCl 5.0 mg, riboflavin 2.0 mg, nicotiamide 75.0 mg, pantothenic acid 2.5 mg, pyridoxine HCl 5.0 mg, and vitamin B_{12} 2.5 mg per ml of mixture

Table 18. Summary of the results obtained from increasing the glucose concentration in the basal diet and substituting the major B-vitamins, folic acid, biotin and inositol for brewer's yeast

Treatment	Mean larval weight	Larvae weighed	Length of larval period	Pupation
	mg	No.	Days	Percent
Control[a]	52.0	23	15.3	93
1[b]	49.1	21	15.1	92
2[c]	42.7	25	15.3	90

[a]Control: Basic diet

b 0.25 ml B-vitamin mixture, 90 mg of inositol, 0.225 mg of folic acid, 0.09 mg biotin and an additional 2 grams of glucose

c Same as [b] except 0.5 ml of B-vitamin mixture was added